REMARKS/ARGUMENTS

Claims 1-2, 4-6, 8-9, 43, 44 are non-obvious in view of Heid, Ohnishi, and First since all the elements of Applicants' claimed invention are not present in the cited references

The Examiner rejected Claims 1-2, 4-6, 8-9, 43, 44 as obvious in view of Heid, Ohnishi, and First. To make this rejection, the Examiner appears to be construing the allele specific probes of Ohnishi as the labeled oligonucleotide probes of Applicants' presently claimed invention (Action at p. 3). Without acquiescing to this construction, and solely for the purposes of expediting prosecution, Applicants here amend independent claim 1 to provide:

> ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest, and each of which is disposed between the ninety-five to one-thousand and thirteen primer sets amplifying a given target gene sequence of interest.

With this amendment, Applicants make clear in the claim language that the ninety-five to one-thousand and thirteen oligonucleotide probes are disposed between the ninety-five to one-thousand and thirteen primer sets. Accordingly, the Examiner's construction that Applicants' labeled oligonucleotide probes are found as the allele specific probes of Ohnishi is not applicable. This element is not taught in any of Heid, Ohnishi, First, or any combination thereof. Since every element of a claimed invention must be present in the cited references in order for a proper 35 USC 103 rejection to apply, this rejection must fall. Further, since the rejected dependant claims all ultimately depend from independent claim 1, they too should not be rejected under 35 USC 103. Applicants note that certain of the Examiner's arguments regarding certain dependant claims are not addressed since they are believed moot in light of Applicants'

independent claim. By not addressing these arguments, Applicants in no way acquiesce to them.

Claims 1-4, 19-28, and 32 are non-obvious in view of Dolganov and First since all the elements of Applicants' claimed invention are not present in the cited references

The Examiner rejected Claims 1-4, 19-28, and 32 as obvious in view of Dolganov and First. To make this rejection, the Examiner appears to be construing the gene specific primers of Dolganov as the labeled oligonucleotide probes of Applicants' presently claimed invention (Action at p. 6). Without acquiescing to this construction, and solely for the purposes of expediting prosecution, Applicants here amend independent claim 1 to provide:

> ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest, and each of which is disposed between the ninety-five to one-thousand and thirteen primer sets amplifying a given target gene sequence of interest.

With this amendment, Applicants make clear in the claim language that the ninety-five to one-thousand and thirteen oligonucleotide probes are disposed between the ninety-five to one-thousand and thirteen primer sets. Accordingly, the Examiner's construction that Applicants' labeled oligonucleotide probes are found as the gene specific primers of Dolganov is not applicable. This element is not taught in any of Dolganov, First, or any combination thereof. Since every element of a claimed invention must be present in the cited references in order for a proper 35 USC 103 rejection to apply, this rejection must fall. Further, since the rejected dependant claims all ultimately depend from independent claim 1, they too should not be rejected under 35 USC 103. Applicants note that certain of the Examiner's arguments regarding certain dependant claims are not addressed since they are believed moot in light of Applicants' independent claim. By not addressing these arguments, Applicants in no way acquiesce to them.

The Combination of References Proposed by the Examiner would not Provide a Reasonable Expectation of Success

The field of molecular biology is considered an uncertain art. In the context of multiplexed PCR, it is generally recognized that increasing the number of target nucleic acids to be analyzed results in a concomitant increase in the number of undesired side reactions. For example, primer dimer formation can rapidly swamp out the desired amplification reactions, thus polluting a PCR with unwanted side products. To further support the above declaration, Applicants earlier submitted a publication by Rudi et al. (hereinafter "Rudi") that corroborates the above characterizations of the knowledge of one of skill in the art. (Rudi et al., Nucleic Acids Research, 2003, Vol. 31, No. 11 e62).

> For example, the Introduction of Rudi begins: DNA amplification techniques, in particular the polymerase chain reaction (PCR) (1), have become key diagnostic tools. Challenges with PCR, however, are still to obtain quantitative information, and to analyse several targets simultaneously. Developments of multiplex PCR are generally limited by the complexity of the amplification reaction. The number of possible primer pair combinations increases arithmetically with the number of primers present in the reaction, and leads to distorting side reactions. These background amplifications together with differences in amplification efficiencies between amplicons represent severe challenges with multiplex PCR (2).

Rudi et al., then go on to describe a new multiplexed method, involving the laborious nuclease-mediated removal of unincorporated primers as a way of minimizing unwanted side reactions. The authors employ their approaches to achieve multiplex reactions of eight-plex, and twelve-plex. The Examiner is invited again to consider Rudi as one illustrative teaching of the difficulties of performing accurate multiplexed PCR, at a time period after Applicants' claimed invention.

Thus, Applicants assert that performing a multiplexed PCR with 95-1013 different primer pairs, along with "ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest, and each of which is disposed between the ninety-five

to one-thousand and thirteen primer sets amplifying a given target gene sequence of interest," would not have been expected reasonably likely to succeed at the time of Applicants' invention.

Applicants were able to achieve not only a highly multiplexed PCR with a large number of primers, but *the multiplexed PCR also contains oligonucleotide probes* (e.g. TaqMan probes). Applicants respectfully submit that one of skill in the art at the time of Applicants' invention would have reasonably concluded that adding oligonucleotide probes to an already highly multiplexed PCR would merely produce a lot of unwanted side products, and simply would not work. The Rudi submission supports this contention.

There is No Motivation to Combine the References Proposed by the Examiner to Produce Applicants Claimed Invention

Applicants claim a highly multiplexed PCR containing a large number (95-1023) of primer pairs, as well as a large number of (95-1023) oligonucleotide probes. Further, Applicants have here further clarified their claim language to provide that each oligonucleotide probe is disposed between the relevant ninety-five to one-thousand and thirteen primer sets amplifying a given target gene sequence of interest. One of skill in the art would have no motivation for doing such a thing, since no way of discriminating 95-1023 oligonucleotide probes would be achievable in the multiplex PCR. That is, 95-1023 different labels don't readily exist that are capable of selective detection by current instrumentation. Thus, there would be no motivation to include them in the reaction. It would be economically wasteful to do so. Further, as discussed earlier, there would no motivation to include so many oligonucleotide probes in the PCR, since one of skill in the art at the time of Applicants' invention would have believed that doing so would merely increase the occurrence of unwanted side products.

Applicants discovered that pooling a large number of single plex quantitative PCR kits (a primer pair and a single oligonucleotide probe in each kit) into a single very large multiplex reaction could be performed, and by using for example low primer concentrations and a limited number of PCR cycles in a first highly multiplexed PCR, highly quantitative results could be obtained in a second PCR. This second PCR can be a single-plex, and can include one of the very same kits (primer pair and

oligonucleotide probe) that were present in the initial pooled collection of kits. When this approach was attempted, highly accurate results were obtained. These results were truly unexpected, since one of skill in the art prior to Applicants' claimed invention would have understood that multiplexed PCR produces unwanted side products, and adding additional nucleic acids (e.g. oligonucleotide probes) to an already complex reaction mixture would surely just make more unwanted side products. Further, oligonucleotide probes are expensive to manufacture; adding them unnecessarily to a reaction would be economically wasteful. Again, there would be no motivation for performing this combination.

Simply put, one of skill in the art of molecular biology and multiplexed PCR, at the time of Applicants' invention, would not have reasonably been motivated to take a highly complicated reaction (a multiplexed PCR) and make it even more complicated (by adding oligonucleotide probes), as is taught by Applicants' presently claimed invention. Performing such a combination would be believed to simply produce a lot of unwanted non-specific side products, and would be considered a wasteful use of expensive-to-manufacture oligonucleotide probes.

One benefit of Applicants' approach is that it allows pre-existing kits (e.g.-single-plex PCR kits containing a primer pair and an oligonucleotide probe) to simply be pooled together to perform the highly multiplexed reaction, and then, those same kits in single-plex form can be used in the second PCR to accurately quantify the target nucleic acid. No additional kits that lack the oligonucleotide probes need to be manufactured. When dealing with thousands and thousands of kits, this provides an enormous manufacturing advantage. Further, the ability to accurately perform highly multiplexed quantitative PCR is unprecedented.

CONCLUDING REMARKS

Applicants here first illustrated that every element of their presently claimed invention was not present in the references cited by the Examiner, and provided claim amendments to further highlight the differences between the claimed invention and the cited art. Alone, this is sufficient to successfully attack the Examiner's obviousness rejection. However, for the sake of expediting prosecution, Applicants went on to show that one of skill in the art would not have had a reasonable expectation that the Examiner's proposed combination would have worked. Applicants also have shown that one of skill in the art would not have had a reason to make the combination in view of the relevant teachings in the art. Thus, for any of these reasons, alone or in combination, the Examiner has not provided a prima facie showing of obviousness. Applicants request that the rejection be withdrawn and the claims allowed.

As a final note, though the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.